

(19)



Europäisches Patentamt

European Patent Office

Office européen des brevets



(11)

EP 1 245 572 A1

(12)

EUROPEAN PATENT APPLICATION

(43) Date of publication:
02.10.2002 Bulletin 2002/40

(51) Int Cl.⁷: **C07J 43/00**, C07J 41/00,
A61K 31/58, A61K 31/57,
A61P 15/08

(21) Application number: **01250108.6**

(22) Date of filing: **26.03.2001**

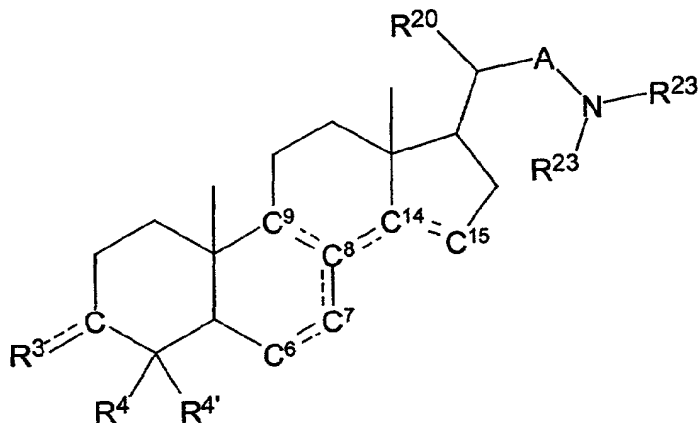
(84) Designated Contracting States:
**AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU
MC NL PT SE TR**
Designated Extension States:
AL LT LV MK RO SI

(71) Applicant: **Schering Aktiengesellschaft**
13353 Berlin (DE)

(72) Inventors:
• **Blume, Thorsten**
16552 Berlin (DE)
• **Esperling, Peter**
12107 Berlin (DE)
• **Hegele-Hartung, Christa**
45470 Mülheim/Ruhr (DE)

(54) **Aminosterol compounds, their use in the preparation of meiosis-regulating medicaments and method for their preparation**

(57) The present invention relates to steroid compounds of general formula I, which may advantageously be employed to stimulate meiosis in human oocytes, the steroid being specifically characterized by amino nitrogen bonded to C¹⁷ of the steroid skeleton via a spacer A.



I

EP 1 245 572 A1

Description

Specification:

[0001] The invention relates to pharmaceutically active steroid compounds, pharmaceutical compositions comprising these compounds, the use of these compounds for the preparation of meiosis regulating medicaments, a method for regulating meiosis and a method for the preparation of the novel steroid compounds.

[0002] Meiosis is the unique and ultimate event of germ cells, on which sexual reproduction is based. Meiosis comprises two meiotic divisions. During the first division, exchange between maternal and paternal genes take place before the pairs of chromosomes are separated into the daughter cells. These contain only half the number (1n) of chromosomes and 2c DNA. The second meiotic division proceeds without a DNA synthesis. This division therefore results in the formation of the haploid germ cells with only 1c DNA.

[0003] The meiotic events are similar in the male and female germ cells, but the time schedule and the differentiation processes, which lead to ova and to spermatozoa differ profoundly. All female germ cells enter the prophase of the first meiotic division early in life, often before birth, but all are arrested as oocytes later in the prophase (dictyate state) until ovulation after puberty. Thus, from early life the female has a stock of oocytes, which is drawn upon until the stock is exhausted. Meiosis in females is not completed until after fertilization, and results in only one ovum and two abortive polar bodies per germ cell. In contrast, only some of the male germ cells enter meiosis from puberty and leave population of germ cells throughout life. Once initiated, meiosis in the male cell proceeds without significant delay and produces four spermatozoa.

[0004] Only little is known about the mechanisms, which control the initiation of meiosis in the male and in the female. New studies indicate that follicular purines, hypoxanthine and adenosine could be responsible for meiotic arrest in the oocyte [S.M. Downs et al., *Dev. Biol.*, **82**, 454-458 (1985); J.J. Epplg. et al., *Dev. Biol.*, **119**, 313-321 (1986); S.M. Downs, *Mol. Reprod. Dev.*, **35**, 82-94 (1993)]. The presence of a diffusible meiosis regulating substance was first described by Byskov et al. in a culture system of fetal mouse gonads [A.G. Byskov et al., *Dev. Biol.*, **52**, 193-200 (1976)]. A meiosis activating substance (MAS) is secreted by the fetal mouse ovary, in which meiosis is ongoing, and a meiosis preventing substance (MPS) is released from the morphologically differentiated testis with resting, non-meiotic germ cells. It was suggested that the relative concentrations of MAS and MPS regulate the beginning, arrest and resumption of meiosis in the male and in the female germ cells [A.G. Byskov et al. in: *The Physiology of Reproduction* (eds. E. Knobil and J.D. Neill), Raven Press, New York (1994)]. Clearly, if meiosis can be regulated, reproduction can be controlled. In a recent article [A.G. Byskov et al., *Nature*, **374**, 559-562 (1995)] the isolation of certain sterols is described that activate oocyte meiosis from bull testes and from human follicular fluid [T-MAS (testes meiosis-activating sterol) and FF-MAS (follicular fluid meiosis-activating sterol): 4,4-dimethyl-5 α -cholesta-8,14,24-trien-3 β -ol].

[0005] It was also demonstrated that micromolar concentrations of synthetic FF-MAS are able to induce resumption of meiosis in a dose-dependent manner in rat oocytes that are arrested by the phosphodiesterase inhibitor IBMX (3-isobutyl-1-methyl xanthine) [C. Hegele-Hartung et al., *Biol. Reprod.*, **64**, 418-424 (2001)]. It was shown that this effect can be observed when CEO (cumulus-enclosed oocytes) and DO (denuded oocytes) are cultured *in vitro* in the presence of FF-MAS.

[0006] Further substances that regulate the meiosis are described in WO 96/00235 A1, WO 96/27658 A1, WO 97/00884 A1, WO 98/28323 A1, WO 98/52965 A1 and WO 00/68245 A1.

[0007] In WO 98/52965 A1 meiosis activating 20-aralkyl-5 α -pregnane derivatives are described.

[0008] In WO 00/68245 A1 steroid compounds are disclosed, which are able to inhibit meiosis such that these compounds are useful as contraceptives in females and males. These compounds are primarily unsaturated cholestan derivatives characterized by a 3 β -hydrogen atom bonded to the C¹⁴ carbon atom of the cholestan skeleton.

[0009] In WO 96/00235 A1 meiosis inducing sterols, being known as intermediates in the biosynthesis of cholesterol, as well as certain structurally related synthetic sterols, are described. These substances have been found to regulate meiosis. Similar to cholesterol these sterols are provided with a side chain on C¹⁷ in the sterol skeleton and further with at least one of a Δ^7 , Δ^8 or $\Delta^8(14)$ double bond.

[0010] In WO 96/27658 A1 a method of stimulating meiosis of a germ cell is disclosed, which comprises administering to the cell *in vivo*, *ex vivo* or *in vitro* an effective amount of a compound, which causes accumulation of an endogenous meiosis activating substance to a level, at which meiosis is induced. Such compounds which cause accumulation of the meiosis activating substance are disclosed to be amphotericin B, aminoguanidine, 3 β ,5 α ,6 β -trihydroxycholestan, melatonin, 6-chloromelatonin and 5-methoxytryptamine as well as other derivatives and agonists thereof. Meiosis activating substances are reported to be *inter alia* 5 α -cholestan-3 β -ol, D-homo-cholesta-8,14-dien-3 β -ol and 22,25-diazacholesterol, 23- and 24-azacholesterol as well as 25-azacholestanol derivatives.

[0011] In WO 97/00884 A1 and in WO 98/28323 A1 substances are described which can be used for stimulating meiosis *in vitro*, *in vivo* or *ex vivo*. The compounds disclosed are hence agonists of naturally occurring meiosis activating substances and may therefore be used in the treatment of infertility which is due to insufficient stimulation of meiosis

in females and males. In this document also some compounds are disclosed which may be antagonists of naturally occurring meiosis activating substances, such that these compounds may be suitable for use as contraceptives. The compounds disclosed *inter alia* comprise 5α -cholest-8-ene- 3β -ols and 5α -cholest-8,14-dien- 3β -ols which *inter alia* may be provided with an amino group in the side chain bonded to C¹⁷ of the cholesterol skeleton, the amino group being bonded to the sterol skeleton via a C₄-spacer. To the amino group C₁ - C₄ alkyl or C₃ - C₆ cycloalkyl are bonded.

[0012] It has been discovered, when using previously described meiosis regulating components, that resumption of meiosis occurs in naked oocytes *in vitro*. However, these compounds were only marginally effective when stimulating meiosis in oocytes surrounded by granulosa cells (CEO = cumulus enclosed oocytes). The disclosure of the above documents is incorporated by reference.

[0013] One object of the present invention is to find substances that are useful for regulating meiosis in females and males, in particular in humans.

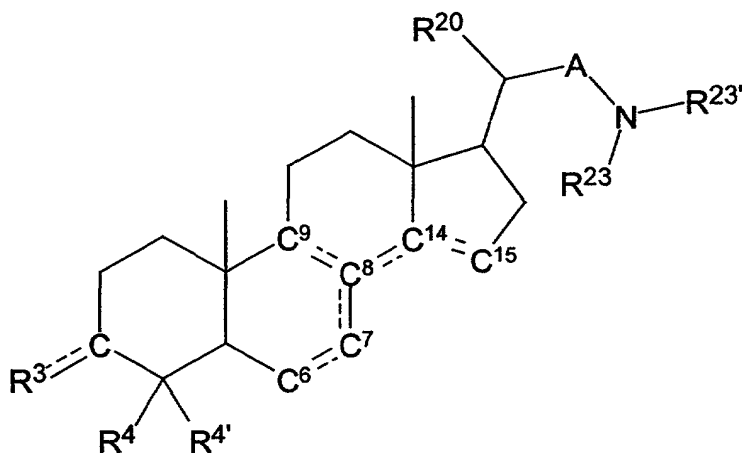
[0014] It is another object of the present invention to provide a novel pharmaceutical composition comprising the novel substances.

[0015] It is another object of the present invention to provide a use of the novel substances for the preparation of a meiosis-regulating medicament.

[0016] It is another object of the present invention to provide a novel method of regulating meiosis.

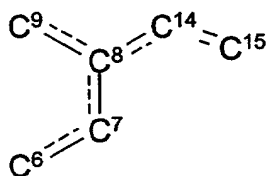
[0017] It is another object of the present invention to provide a method for the preparation of the novel substances.

[0018] According to the present invention steroid compounds of general formula I may advantageously be employed in regulating meiosis in females and males, especially in humans, wherein the term "regulating meiosis" is used to indicate that the compounds according to the present invention are able to stimulate meiosis in human oocytes, such that these compounds which are agonistic analogues of a naturally occurring meiosis activating substance, can be used in the treatment of infertility which is due to insufficient stimulation of meiosis in females and males:



I

wherein in the moiety

**IA**

each bond between C⁶ and C⁷, between C⁷ and C⁸, between C⁸ and C⁹, between C⁸ and C¹⁴ and between C¹⁴ and C¹⁵, independently, may be a single bond or a double bond, with the proviso that each carbon atom C⁶, C⁷, C⁸, C⁹, C¹⁴ and C¹⁵ is bonded to each neighbouring C atom by a single bond or at the most by one double bond, CR³ is

a) C=O or

b) CH-OR^{3'}, wherein R^{3'} is selected from the group comprising hydrogen, unsubstituted or substituted, linear or branched C₁ - C₁₀ alkyl and C(O)-R^{3''}, bonded to the CH-O moiety via the C(O) moiety, wherein R^{3''} is selected from the group comprising

i) substituted or unsubstituted, linear or branched C₁ - C₁₀ alkyl,

ii) substituted or unsubstituted, linear or branched C₁ - C₁₀ fluoro alkyl,

iii) unsubstituted or substituted C₁ - C₁₀ aryl,

iv) unsubstituted or substituted C₁ - C₁₀ heteroaryl,

v) unsubstituted or substituted, linear or branched C₁ - C₁₀ alkyloxy and

vi) unsubstituted or substituted, linear or branched C₁ - C₁₀ alkylamino, or

c) CH-SO₂-R^{3''} or C=NOR^{3''}, wherein R^{3''} has the same meaning as above, or

d) CH-O-R^{3'''}, wherein R^{3'''} is unsubstituted or substituted, linear or branched alkylene and forms a cyclic ether both with the C atom of the steroid skeleton and the O atom, or

e) a cyclic ring structure with the C³ atom, wherein R³ is unsubstituted or substituted, linear or branched C₂ - C₁₀ alkylene, or

f) CH-Hal, wherein Hal is F, Cl, Br or I,

R⁴, R^{4'} and R²⁰, independently, are selected from the group comprising hydrogen and unsubstituted or substituted, linear or branched C₁ - C₄ alkyl,

R²³ and R^{23'}, independently, are selected from the group, comprising hydrogen, unsubstituted or substituted, linear or branched C₁ - C₈ alkyl and unsubstituted or substituted, linear or branched C₁ - C₈ alkenyl and unsubstituted or substituted, linear or branched C₁ - C₈ alkyl, at least one of the alkyl carbon atoms being substituted by any of O, N and S and unsubstituted or substituted, linear or branched C₁ - C₈ alkenyl, at least one of the alkenyl carbon atoms being substituted by any of O, N and S and unsubstituted or substituted, linear or branched C₆ - C₁₀ aryl, or

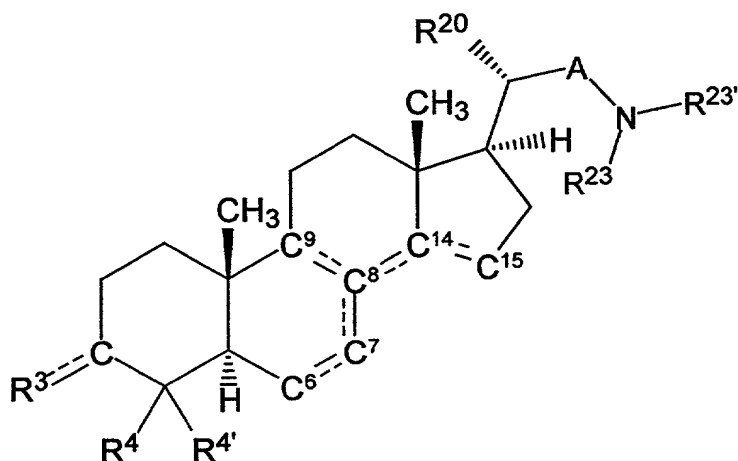
R²³ and R^{23'} together form an unsubstituted or substituted, linear or branched C₁ - C₇ alkylene spacer or an unsubstituted or substituted, linear or branched C₁ - C₇ alkylene spacer, at least one of the alkylene carbon atoms being substituted by any of O, N and S,

A is a single bond or an unsubstituted or substituted methylene or ethylene spacer.

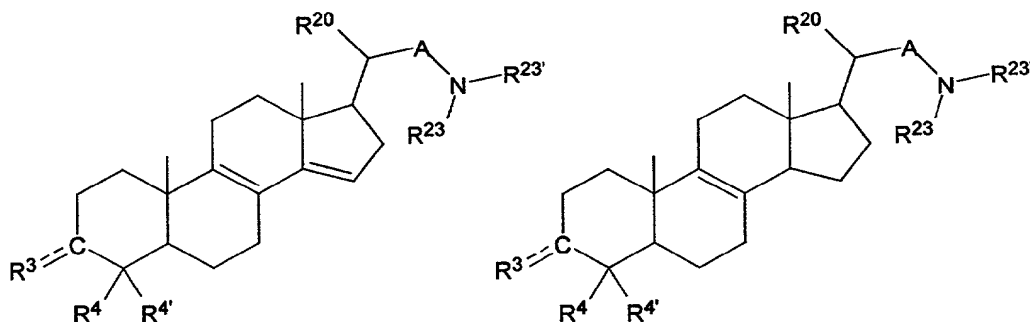
[0019] All indications to C_n alkyl, C_n fluoroalkyl, C_n alkyloxy, C_n alkylamino, C_n cycloalkyl, C_n alkylene, C_n alkenyl, C_n aryl, C_n heteroaryl and the like relate to radicals with n carbon atoms in the moiety, the number of n carbon atoms including all carbon atoms in side chains of e.g. branched radicals. Further aryl also represents alkylaryl, heteroaryl also represents alkylheteroaryl, and cycloalkyl also represents alkylcycloalkyl.

[0020] The novel steroid compounds have a number of chiral centers such that these compounds exist in several isomeric forms. All these isomeric forms are within the scope of the present invention unless otherwise noted.

[0021] A steroid compound with the following general formula is preferred:

**IB**

[0022] Especially the Δ^8 -pregnene derivatives and the $\Delta^{8,14}$ -pregnadien derivatives are useful as pharmaceutically active steroid compounds for regulating meiosis, i.e. compounds with following general formulae:



[0023] It was surprisingly found that the compounds according to the present invention have a strong meiosis stimulating effect in oocytes, especially in CEO, though these compounds are structurally highly different to sterol FF-MAS. In this respect the compounds of this invention are superior to this formerly described meiosis-regulating substance [e.g.: A.G. Byskov et al., *Nature*, 374, 559-562 (1995)]. Preferred compounds of general formula I are those, which induce the germinal vesicle breakdown by at least 40 %, preferably at least 60 % and especially at least 80 % when tested in an oocyte test as described in example 8.

[0024] The compounds according to the present invention are superior to the formerly described compounds in a second aspect: Whereas FF-MAS is not able to induce maturation in a follicle culture system, the compounds of the present invention are able to activate meiosis in this situation.

[0025] For this reason the novel steroid compounds can e.g. be employed for *in-vitro* and *in-vivo* fertilization of mammals, especially of humans.

[0026] The outstanding properties of the novel compounds are mainly attributed to the amino group in the side chain linked to the C¹⁷ carbon atom in the steroid skeleton via a C₁ - C₃ alkyl spacer.

[0027] Especially preferred are compounds, wherein the moiety CR³ is CH-OH, in particular a 3 β -hydroxy radical

bonded to the C³ atom of the steroid skeleton. The moiety may also be CH-O-C(O)-R^{3'} (= CH-O-R^{3'}, wherein R^{3'} is C(O)-R^{3''}), wherein R^{3''} is defined as before. In particular R³ may be an ester radical of a monocarboxylic acid, a dicarboxylic acid, of an inorganic acid or of any other acid, bonded to the C³ atom of the steroid skeleton. Especially for R³ being an ester radical of a dicarboxylic acid R^{3'} may be (CH₂)_n-COOH, wherein n = 1, 2, 3, 4, 5 or 6. The ester radical may also be formed from an inorganic acid such as phosphoric acid, sulfuric acid and sulphamic acid, further from a monocarboxylic acid such as acetic acid, propionic acid, n-butyric acid, pivalic acid, benzoic acid, nicotinic acid and isonicotinic acid. In particular the ester radical may be formed from a dicarboxylic acid, such as from succinic acid and glutaric acid.

[0028] Further steroid compounds according to the present invention may also include derivatives, in which C-O-R³ represents a cyclic ether including the C³ atom of the steroid skeleton.

[0029] R³ may also form a cyclic ring structure together with the C³ atom, R³ being unsubstituted or substituted, linear or branched C₂ - C₁₀ alkyl. E.g. CR³ may be a cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl radical. It may also represent an unsaturated cyclic ring structure such as cyclopropenyl, cyclobutenyl, cyclopentenyl and cyclohexenyl. The ring structure may also be substituted by any of halogen, hydroxy, alkoxy, aryloxy and the like.

[0030] Substances according to the present invention may advantageously also be compounds, in which R³ is selected from the group comprising fluoromethyl, aryl, heteroaryl and (CH₂)_n-COOH, wherein n = 1, 2, 3, 4, 5 or 6, especially compounds, in which R^{3'} (= C(O)-R^{3''}) is acetyl, propionyl, pivaloyl, butanoyl, benzoyl, nicotinyl, isonicotinyl, hemi glutaroyl, butylcarbamoyl, phenylcarbamoyl and *tert*-butoxycarbonyl. In a particularly preferred steroid compound R³ may be hemi succinoyl.

[0031] Further in the novel steroid compounds R⁴ and R^{4'}, independently, are preferably hydrogen or a linear or branched C₁ - C₄ alkyl group, i.e. methyl, ethyl, propyl and butyl, and especially methyl.

[0032] Further R⁴ and R^{4'}, independently, may also be C₁ - C₄ alkyl, substituted by halogen, hydroxy, alkoxy or aryloxy.

[0033] R²⁰ is preferably hydrogen or linear or branched C₁ - C₄ alkyl, i.e. methyl, ethyl, propyl and butyl. R²⁰ is especially methyl.

[0034] R²³ and R^{23'}, independently, may specifically comprise hydrogen or an C₁ - C₈ alkyl group, such as methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *isobutyl*, *tert*-butyl, *n*-pentyl, *iso*-pentyl, *tert*-pentyl, *neo*-pentyl, further hexyl and cyclohexyl and the like. Further R²³ and R^{23'}, independently, may also comprise an C₁ - C₈ alkenyl group, i.e. an unsaturated alkyl group, e.g. vinyl, allyl, *iso*-propenyl and prenyl, further C₆ - C₁₀ aryl, such as phenyl and 1-naphthyl, this group also comprising alkylaryl, being bonded via the aryl moiety or via the alkyl moiety to the nitrogen atom, e.g. benzyl and tolyl. R²³ and R^{23'} may preferably be alkyl and alkenyl, being substituted by at least one radical, selected from the group, comprising linear or branched C₁ - C₄ alkyl and C₁ - C₄ alkoxy. The phenyl and 1-naphthyl radical may also be substituted by halogen, C₁ - C₄ alkoxy, hydroxy or C₁ - C₄ alkyl, including the fluoroalkoxy and fluoroalkyl derivatives. Further R²³ and R^{23'}, independently, may further comprise e.g. 4-hydroxy phenyl, 4-methoxy phenyl, 2,4,6-trimethyl phenyl, 2,4-dichloro phenyl, 4-fluoro phenyl, 4-trifluoromethyl phenyl and 2-pentafluoroethyl phenyl.

[0035] Further R²³ and R^{23'}, independently, may also represent alkyl and alkenyl, at least one of the alkyl and alkenyl carbon atoms, respectively, being substituted by any of O, N and S, e.g. methoxymethylen, methoxyethylen, methoxypropylen, ethoxypropylen and the like.

[0036] R²³ and R^{23'} together may also form a heterocyclic ring structure bonded to the side chain together with the nitrogen atom in the side chain, the nitrogen atom being linked to the C¹⁷ carbon atom of the steroid skeleton via the spacer A. This heterocyclic ring structure may especially be a compound being selected from the group, comprising piperidin-1-yl, morpholin-4-yl, piperazin-1-yl, pyrrolidin-1-yl, pyridin-1-yl, chinolin-1-yl, isochinolin-1-yl, pyridazin-1-yl, pyrimidin-1-yl, pyrazin-1-yl, pyrrol-1-yl, indol-1-yl, chinoxalin-1-yl, pyrazol-1-yl, imidazol-1-yl, thiazol-1-yl and oxazol-3-yl ring structures and substituted derivatives thereof. Preferred ring structures are the saturated radicals, namely piperidin-1-yl, morpholin-4-yl, piperazin-1-yl and pyrrolidin-1-yl. The heterocyclic ring structures may be substituted with alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, alkylcycloalkyl, aryl, alkylaryl, hydroxy, alkoxy, alkylcycloalkoxy, alkyloxycycloalkyl, alkylaryloxy, alkyloxyaryl, halogen and acyl.

[0037] The nitrogen atom of the heterocyclic radical preferably is not bonded directly but via A to the C¹⁷ atom, A being a single bond or an unsubstituted or substituted methylen or ethylen spacer, such as e.g. (unsubstituted) methylen and (unsubstituted) ethylen and further *iso*-propylen, *tert*-butylen and the like. Preferably A is methylen.

[0038] Especially preferable are compounds, in which R³ is hydroxy or hemi succinate ester, in which R⁴, R^{4'} and R²⁰ are each methyl and in which the ring structure including the amino nitrogen atom is a morpholin-4-yl, piperidin-1-yl, 3,3-dimethylpiperidin-1-yl, 4,4-dimethylpiperidin-1-yl, 4-methylpiperazin-1-yl, 4-phenylpiperazin-1-yl, pyrimidin-2-yl and pyrrolidin-1-yl or in which R²³ and R^{23'} are hydrogen, 2,2-dimethylethylen, 2-methoxyethylen and 2-methoxypropylen.

[0039] Hydrogen atoms may be bonded to all other skeleton C atoms of the steroid compounds, i.e. to C¹, C², C⁶, C⁷, C⁸, C⁹, C¹¹, C¹², C¹³, C¹⁴, C¹⁵ and C¹⁶.

[0040] Preferably pharmaceutically acceptable compounds of the present invention are salts of steroid compounds

of general formula I. Examples of these salts are listed in *Journal of Pharmaceutical Science*, 66, 2 et seq. (1977). Examples of such salts include salts of organic acids such as formic acid, fumaric acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, succinic acid, malic acid, tartaric acid, citric acid, benzoic acid, salicylic acid, methane sulphonic acid and the like. Suitable inorganic acids to form pharmaceutically acceptable salts include hydrochloric acid, hydrobromic acid, sulphuric acid, phosphoric acid and the like.

[0041] The following compounds according to the present invention are especially preferred:

- 1) (20S)-20-[(3,3-dimethylpiperidin-1-yl)methyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol,
- 2) (20S)-20-[(piperidin-1-yl)methyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol,
- 3) (20S)-20-[(4,4-dimethylpiperidin-1-yl)methyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol,
- 4) (20S)-20-[(4-methylpiperazin-1-yl)methyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol,
- 5) (20S)-20-[(4-phenylpiperazin-1-yl)methyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol,
- 6) (20S)-20-[(morpholin-4-yl)methyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol,
- 7) (20S)-20-[(4-(pyrimidin-2-yl)piperazin-1-yl)methyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol,
- 8) (20S)-20-[(pyrrolidin-1-yl)methyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol,
- 9) (20S)-20-[(3,3-dimethylpiperidin-1-yl)methyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol hemisuccinate,
- 10) (20S)-20-[N-(3-methoxypropyl)aminomethyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol,
- 11) (20S)-20-aminomethyl-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol,
- 12) (20S)-20-[N,N-di-(2-methoxyethyl)aminomethyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol,
- 13) (20S)-20-[N-(2,2-dimethylethyl)aminomethyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol.

[0042] The structural formulae of these steroid compounds are shown in **Fig. 2A**, **Fig. 2B** and **Fig. 2C**.

[0043] A further object of the present invention are pharmaceutical compositions comprising at least one steroid compound of general formula I and at least one pharmaceutically acceptable excipient well known in the art, e.g. at least one carrier, diluent, absorption enhancer, preservative, buffer, agent for adjusting the osmotic pressure and rheology of the medicament if it will be liquid, surfactant, solvent, tablet disintegrating agent, micro capsules, filler, slip additive, colorant, flavour and other ingredient. These substances are conventionally used in the art.

[0044] Examples for solid carriers are magnesium carbonate, magnesium stearate, dextrin, lactose, sugar, talkum, gelatin, pectin, starch, silica gel, tragacanth, methylcellulose, sodium carboxymethyl cellulose, low melting waxes and cacao butter.

[0045] Liquid compositions include sterile solutions, suspensions and emulsions, which may be administered e.g. orally by nasal administration or as an ointment. Such liquid compositions may also be suitable for injection or for use in connection with *ex vivo* or *in vivo* application. For oral administration the liquid may contain a pharmaceutically acceptable oil and/or lipophilic, surfactant and/or solvent which is miscible with water. In this connection reference is made to WO 97/21440 A1.

[0046] Liquid compositions may also contain other ingredients, which are conventionally used in the art, some of which are mentioned in the list above. Further a composition for transdermal administration of a compound of the present invention may be provided in the form of a patch. A composition for nasal administration may be provided in the form of a nasal spray in liquid or in powder form.

[0047] In order to enhance bioavailability of the steroid compound these compounds may also be formulated as cyclodextrin chlates. For this purpose the compounds are compounded with α -, β - or γ -cyclodextrin or derivatives thereof.

[0048] Salves, ointments, lotions and other liquids to be administered externally must be in a condition such that the steroid compounds of the present invention may be delivered to the subject in need of regulation of meiosis in sufficient quantity. For this purpose the medicament contains excipients for regulating the rheology of the medicament, surfactants, preservatives, solvents, diluents, substances for enhancing skin permeation ability, further flavours and protective skin substances such as conditioners and moisture regulators.

[0049] The medicament may also contain further active agents to enhance or regulate the effectiveness of the steroid compounds or to produce other desired effects of the medicament.

[0050] For parenteral administration the steroid compounds may be dissolved or suspended in a pharmaceutically acceptable diluent. Oils are very often used in combination with solvents, surfactants, suspension or emulsion agents, e.g. olive oil, peanut oil, soybean oil, castor oil and the like. For the preparation of an injectable medicament any liquid carrier may be employed. These liquids often also contain agents for the regulation of the viscosity thereof as well as agents for regulating isotonicity of the liquid.

[0051] The steroid compound according to the present invention may further be administered as a injectable depot or as an implantate, which may e.g. be administered subcutaneously, such that delayed release of the steroid compounds is made possible. For this purpose various techniques may be employed, e.g. administration of depots, which include a membrane containing the active compound, or of slowly dissolving depots. Implantates may e.g. contain biologically

degradable polymers or synthetic silicones as inert material.

[0052] The dose of a steroid compound to be used will be determined by a physician and will depend *inter alia* on the particular steroid compound employed, on the route of administration and on the purpose of the use. In general, the compositions of the present invention are prepared by intimately bringing into association the active compound

with the liquid or solid auxiliary ingredients and then, if necessary, shaping the product into the desired formulation.

[0053] Usually not more than 3000 mg, preferably not more than 350 mg, and in some preferred instances not more than 30 mg of the steroid compounds are to be administered to mammals, e.g. to humans, per day.

[0054] The present invention also relates to the use of the steroid compounds of general formula I for the preparation of a meiosis-regulating medicament.

[0055] The present invention also relates to a method of regulating meiosis comprising administering to a subject in need of such a regulation an effective amount of at least one steroid compound of general formula I.

[0056] The route of administration of compositions containing a compound of the present invention may be any route, which effectively transports the active steroid compound to its site of action.

[0057] Thus, when the steroid compounds are to be administered to a mammal, they are conveniently provided in the form of a pharmaceutical composition, which comprises at least one steroid compound according to the present invention in connection with a pharmaceutically acceptable carrier. For oral use, such compositions are preferably in the form of tablets or capsules.

[0058] The present invention also relates to a method for the preparation of steroid compounds of general formula I, wherein R⁴ and R^{4'} are unsubstituted or substituted, linear or branched C₁ - C₄ alkyl and not hydrogen:

[0059] The aforementioned steroid compounds may be synthesized analogously with the preparation of known compounds. Hence, synthesis of the steroid compounds of formula I may follow the well established synthetic pathways described in the comprehensive sterol and steroid literature. The following literature may be used as the key source for synthesis: L.F. Fieser & M. Fieser: *Steroids*, Reinhold Publishing Corporation, N.Y., 1959; *Rood's Chemistry of Carbon Compounds* (ed. S. Coffrey): Elsevier Publishing Company, 1971; and especially *Dictionary of Steroids* (eds. R.A. Hill, D.N. Kirk, H.L.J. Makin and G.M. Murphy), Chapman & Hall. The last one contains an extensive list of citations to the original papers covering the period up to 1990.

[0060] Particularly, the steroid compounds may be synthesized e.g. according to the general procedure, comprising

- a. starting from (20S)-20-hydroxymethyl-pregn-4-en-3-one,
- b. introducing two alkyl groups in position 4 by alkylation,
- c. reducing the keto group to a hydroxy group,
- d. introducing a Δ^7 double bond by bromination/dehydrobromination,
- e. isomerizing the dien $\Delta^{5,7}$ to the dien $\Delta^{8,14}$ by heating in the presence of acid,
- f. oxidizing the 17-hydroxy group to an aldehyde group and
- g. reductively aminizing the aldehyde group.

[0061] The corresponding synthesis scheme is shown in **Fig. 1**. According to this, first the hydroxy group in the side chain of (20S)-20-hydroxymethyl-pregn-4-en-3-one **1** is protected as a silyl ether, e.g. as a triisopropylsilyl (TIPS) ether resulting in compound **2**. In order to produce compound **3** two methyl groups are introduced via alkylation with methyl iodide in the presence of a base like potassium *tert*-butoxide in position 4 of the steroid skeleton. In the next step the 3-keto group is reduced with a common reducing agent such as lithium aluminiumhydride or sodium borohydride. The resulting alcohol **4** is then protected e.g. as an acetate (compound **5**). A second double bond is afterwards introduced via a bromination-dehydrobromination sequence. The resulting $\Delta^{5,7}$ -dien system in compound **6** is then isomerized to the $\Delta^{8,14}$ -dien system via heating in the presence of hydrochloric acid to obtain compound **7**. In this acid catalyzed step both hydroxyl groups are deprotected and diol **7** can be obtained. Moderately selective oxidation of the hydroxyl group in the side chain with Dess-Martin periodinane results in aldehyde **8**, which serves as a central intermediate to introduce different amines in the side chain via reductive amination. For this purpose different reducing agents like sodium borohydride or tris-(acetoxymethyl) borohydride may be used. As a result the steroid compounds **9** according to the present invention are obtained.

[0062] Examples are given to more detailedly describe the present invention.

Example 1: (20S)-20-[(piperidin-1-yl)methyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol (Compound No. 2)

a) (20S)-20-[(triisopropylsilyloxy)methyl]-pregn-4-en-3-one

[0063] To a solution of 30 g of (20S)-20-[(hydroxymethyl)-pregn-4-en-3-one and 13.5 g imidazole in 300 ml dichloromethane 26 ml of triisopropylsilylchloride were added dropwise at room temperature. The reaction mixture was stirred for 20 hours at the same temperature and then poured into water. The aqueous layer was extracted with ethyl

EP 1 245 572 A1

acetate. The organic layers were combined, washed with brine, dried over sodium sulfate, filtered and concentrated under reduced pressure to give 45.4 g of crude (20S)-20-(((triisopropylsilyl)oxy)methyl)-pregn-4-en-3-one as a brown oil, which was used without further purification.

5

MS (CI+): 487 (M + H)

b) (20S)-4,4-dimethyl-20-(((triisopropylsilyl)oxy)methyl)-pregn-5-en-3-one

10 **[0064]** A solution of 45.4 g of crude (20S)-20-(((triisopropylsilyl)oxy)methyl)-pregn-4-en-3-one in 320 ml tetrahydrofuran was added to a solution of 42.3 g potassium *tert*-butylate in 950 ml *tert*-butanol at a temperature of 50°C. The mixture was stirred for 10 minutes at the same temperature. Then 50 ml methyl iodide were added and stirring was continued for one hour. The reaction mixture was poured into water and extracted with ethyl acetate. The organic layers were combined, washed with brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography to give 27.3 g (20S)-4,4-dimethyl-20-(((triisopropylsilyl)oxy)methyl)-pregn-5-en-3-one as a pale yellow solid.

MS (CI+): 515 (M + H)

20

c) (20S)-4,4-dimethyl-20-(((triisopropylsilyl)oxy)methyl)-pregn-5-en-3β-ol

[0065] To a solution of 27.3 g (20S)-4,4-dimethyl-20-(((triisopropylsilyl)oxy)methyl)-pregn-5-en-3-one in 500 ml tetrahydrofuran 1.24 g of lithiumaluminum hydride were added cautiously in small portions at room temperature. The reaction mixture was stirred for one hour and then cooled to 0°C. 2.5 ml water, 2.5 ml of a 1 N sodium hydroxide solution and 7.5 ml of water were added successively. The mixture was filtered over celite. The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography to give 18.2 g (20S)-4,4-dimethyl-20-(((triisopropylsilyl)oxy)methyl)-pregn-5-en-3β-ol as a pale yellow solid.

30

MS (CI+): 517 (M + H)

d) (20S)-4,4-dimethyl-20-(((triisopropylsilyl)oxy)methyl)-pregn-5-en-3β-ol acetate

35 **[0066]** To a solution of 18.2 g (20S)-4,4-dimethyl-20-(((triisopropylsilyl)oxy)methyl)-pregn-5-en-3β-ol in 175 ml pyridine 6.24 ml of acetic anhydride were added at room temperature. The reaction mixture was stirred for 20 hours and then poured into an ice/hydrochloric acid mixture. This was extracted with ethyl acetate. The organic layers were combined, washed with brine, dried over sodium sulfate, filtered and concentrated under reduced pressure to give 16.2 g (20S)-4,4-dimethyl-20-(((triisopropylsilyl)oxy)methyl)-pregn-5-en-3-one acetate as a white solid, which was used without further purification.

40

MS (CI+): 559 (M+H)

e) (20S)-4,4-dimethyl-20-(((triisopropylsilyl)oxy)methyl)-pregna-5,7-dien-3β-ol acetate

[0067] To a solution of 16.2 g (20S)-4,4-dimethyl-20-(((triisopropylsilyl)oxy)methyl)-pregn-5-en-3β-ol acetate in a mixture of 100 ml benzene and 100 ml hexane 4.93 g 1,3-dibrom-5,5-dimethyl-hydantoin were added in portions at 70°C. After 30 minutes the mixture was cooled to 0°C and filtered. The filtrate was evaporated in vacuo.

50 **[0068]** To the resulting residue 160 ml toluene and 7.8 ml 2,4,6-trimethylpyridine were added. The mixture was refluxed for 2.5 hours. After cooling the reaction mixture was washed with 1 N hydrochloric acid, saturated sodium bicarbonate solution and brine. The organic layer was dried over sodium sulfate, filtered and evaporated in vacuo. The residue was purified by column chromatography to give 12.5 g (20S)-4,4-dimethyl-20-(((triisopropylsilyl)oxy)methyl)-pregna-5,7-dien-3β-ol acetate as a white solid.

55

MS (CI+): 557 (M + H)

f) (20S)-4,4,20-trimethyl-pregna-8,14-dien-3 β ,21-diol

[0069] A mixture of 16.1 g (20S)-4,4-dimethyl-20-[[[(triisopropylsilyl)oxy)methyl]-pregna-5,7-dien-3 β -ol acetate, 210 ml ethanol, 28 ml benzene and 28 ml concentrated hydrochloric acid was refluxed for 6 hours. After cooling the mixture was poured into saturated sodium bicarbonate solution, extracted with ethyl acetate and washed with brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was recrystallized from dichloromethane and methanol to give 4.48 g (20S)-20-hydroxy-4,4,20-trimethyl-pregna-8,14-dien-3 β -ol.

MS (EI+): 358 (M)

g) (20S)-3 β -hydroxy-4,4,20-trimethyl-pregna-8,14-dien-21-al

[0070] To a solution of 1 g (20S)-4,4,20-trimethyl-pregna-8,14-dien-3 β ,21-diol in 10 ml dichloromethane 5.4 ml of a 0.5 M Dess-Martin-Periodinane-solution were added at room temperature. The mixture was stirred for one hour, poured into saturated sodium bicarbonate solution, extracted with ethyl acetate and washed with brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography to give 230 mg (20S)-3 β -hydroxy-4,4,20-trimethyl-pregna-8,14-dien-21-al as a white solid.

MS (EI+): 356 (M)

h) (20S)-20-[(piperidin-1-yl)methyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol

[0071] 38 mg sodium tris(acetoxy)borohydride were added to a solution of 42 mg (20S)-3 β -hydroxy-4,4,20-trimethyl-pregna-8,14-dien-21-al and 10 μ l piperidine in 3 ml tetrahydrofuran at room temperature. The mixture was stirred for two hours, poured into saturated sodium bicarbonate solution, extracted with ethyl acetate and washed with brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography to give 15 mg (20S)-20-[(piperidin-1-yl)methyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol as a white solid.

MS (EI+): 425 (M)

Example 2: (20S)-20-[(3,3-dimethylpiperidin-1-yl)methyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol (Compound No. 9)

[0072] (20S)-3 β -hydroxy-4,4,20-trimethyl-pregna-8,14-dien-21-al was treated with 3,3-dimethyl-piperidine and sodium tris(acetoxy)borohydride as described in example 1h). (20S)-20-[(3,3-dimethylpiperidin-1-yl)methyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol was isolated as a white solid.

MS (EI+): 453 (M)

Example 3: (20S)-20-[(4-phenylpiperazin-1-yl)methyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol (Compound No. 5)

[0073] (20S)-3 β -hydroxy-4,4,20-trimethyl-pregna-8,14-dien-21-al was treated with N-phenyl-piperazine and sodium tris(acetoxy)borohydride as described in example 1h). (20S)-20-[(4-phenylpiperazin-1-yl)methyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol was isolated as a white solid.

MS (EI+): 502 (M)

Example 4: (20S)-20-[(morpholin-4-yl)methyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol (Compound No. 6)

[0074] (20S)-3 β -hydroxy-4,4,20-trimethyl-pregna-8,14-dien-21-al was treated with morpholine and sodium tris(acetoxy)borohydride as described in example 1h). (20S)-20-[(morpholin-4-yl)methyl]-4,4-dimethyl-5 α -pregna-8,14-dien-

3 β -ol was isolated as a white solid.

MS (EI+): 427 (M)

Example 5: (20S)-20-[(4-(pyrimidin-2-yl)piperazin-1-yl)methyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol (Compound No. 7)

[0075] (20S)-3 β -hydroxy-4,4,20-trimethyl-pregna-8,14-dien-21-al was treated with N-(pyrimidin-2-yl)piperazine and sodium tris(acetoxy)borohydride as described in example 1h). (20S)-20-[(4-(pyrimidin-2-yl)piperazin-1-yl)methyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol was isolated as a white solid.

MS (EI+): 504 (M)

Example 6: (20S)-20-[(pyrrolidin-1-yl)methyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol (Compound No. 8)

[0076] (20S)-3 β -hydroxy-4,4,20-trimethyl-pregna-8,14-dien-21-al was treated with pyrrolidin and sodium tris(acetoxy)borohydride as described in example 1h). (20S)-20-[(pyrrolidin-1-yl)methyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol was isolated as a white solid.

MS (EI+): 411 (M)

Example 7: (20S)-20-[N-(3-methoxypropyl)aminomethyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol (Compound No. 10)

[0077] (20S)-3 β -hydroxy-4,4,20-trimethyl-pregna-8,14-dien-21-al was treated with 3-methoxypropylamine and sodium tris(acetoxy)borohydride as described in example 1h). (20S)-20-[N-(3-methoxypropyl)aminomethyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol was isolated as a white solid.

MS (EI+): 429 (M)

Example 8: Testing of meiosis-activating substances in the mouse oocyte assay

Animals

[0078] Oocytes were obtained from immature female mice (C57Bl/6J x DBA/2J F1-hybrids, Bomholtgaard, Denmark) weighing 13 - 16 grams, that were kept under controlled lighting and temperature. The mice received an intra-peritoneal injection of 0.2 ml gonadotropins (containing 10 IU PMSG, pregnant mare serum gonadotropin, Sigma Cat. No. G-4877) and 48 hours later the animals were killed by cervical dislocation.

Collection and culture of oocytes

[0079] The ovaries were dissected out and the oocytes were isolated in Hx-medium (see below) under a stereo microscope by manual rupture of the follicles using a pair of 27 gauge needles. Spherical, cumulus enclosed oocytes (CEO) displaying an intact germinal vesicle (GV) were placed in α -minimum essential medium (α -MEM without ribonucleosides, Gibco BRL, Cat.No. 22561) supplemented with 3 mM hypoxanthine (Sigma Cat. No. H-9377), 8 mg/ml Human Serum Albumin (HSA, State Serum Institute, Denmark), 0,23 mM pyruvate (Sigma, Cat. No. S-8636), 2 mM glutamine (Flow Cat. No. 16-801), 100 IU/ml penicillin and 100 μ g/ml streptomycin (Flow, Cat No. 16-700). This medium was designated Hx-medium.

[0080] The oocytes were rinsed three times in Hx-medium and cultured in 4-well multidishes (Nunc, Denmark) in which each well contained 0.4 ml of Hx-medium and approx. 25 oocytes. One control (i.e. approx. 25 oocytes cultured in Hx-medium with no addition of test compound) was always run simultaneously with the test cultures, which were made with different concentrations of the compounds to be tested. The cultures were performed at 37°C and 100 % humidity with 5 % CO₂ in air. The culture time was 22 hours.

Examination of oocytes

[0081] Oocytes arrested in meiosis are characterised by an intact nucleus with a prominent nucleolus, known as germinal vesicle (GV). Upon reinitiation of meiosis the nucleolus and the nuclear envelope disappear and this is characterised by a breakdown of the GV, which then is called germinal vesicle breakdown (GVB). Some hours later the oocyte complete a reductional division and elicit the first so called polar body (PB).

[0082] By the end of the culture period, the number of oocytes with germinal vesicle (GV) or germinal vesicle breakdown (GVB) and those with polar body (PB) was counted using a stereo microscope or an inverted microscope with differential interference contrast equipment. The percentage of oocytes with GVB per total number of oocytes and the percentage of oocytes with PB per total number of oocytes was calculated in the test cultures and compared to the control culture.

Table 1

Activation of meiosis in cumulus enclosed mouse oocytes				
Compounds	Oocytes [n]			Activation [%]
	GV	GVB	PB	GVB+PB
Control (Hx)	19	1	4	21
10 μ M FF-MAS	13	6	5	46
0,1 μ M compound example 1	14	7	2	39
1 μ M compound example 1	6	12	6	75
10 μ M compound example 1	1	14	7	95
Hx = Hypoxanthine GV = germinal vesicle GVB = germinal vesicle breakdown PB = polar bodies n = number of oocytes				

Example 9: Testing of meiosis-activating substances in the mouse follicular culture systemAnimals

[0083] Follicles were obtained from 19 - 21 day-old immature female mice (C57B1/6J x CBA/J), that were kept under controlled lighting and temperature.

Collection of serum and culture of follicles

[0084] Animals were anesthetized with ether, and blood was collected by means of eye extraction. After clotting, blood was centrifuges for 15 min at 4000 x g, and serum was collected and stored at -20°C until use.

[0085] Ovaries were removed and placed in Leibovitz L-15 medium (Gibco Cat No. 41300) supplemented with 1 mmol glutamine l⁻¹, 3 mg BSA ml⁻¹, 5 μ g human transferrin ml⁻¹ (without iron), 5 μ g insulin ml⁻¹ (culture-grade chemicals, Sigma, St. Louis, MO) at 37°C.

[0086] Preantral follicles with a diameter of 170-190 μ m were isolated mechanically with two 27-gauge needles attached to 1 ml syringe. They were placed and washed thereafter (3 times) in 4-well culture plates (Nunc, Denmark) in α -minimum essential medium (α -MEM; Gibco Cat No. 11900) supplemented with 2 mmol glutamine l⁻¹, 10 μ g transferrin ml⁻¹, 10 μ g insulin ml⁻¹ and with 3 mg BSA ml⁻¹.

[0087] Follicles of 170 - 190 μ m with normal morphological appearance, i.e. a central spherical oocyte, high density of granulosa cells, and a theca layer enclosing the entire follicle, were selected and individually cultured in 96-well culture plate (Nunc, Denmark) inserts with 40 μ l α -MEM culture medium supplemented with 50 μ l immature mouse serum ml⁻¹, 5 μ g insulin ml⁻¹, 2 mmol glutamine l⁻¹, 10 μ g human transferrin ml⁻¹ and 0.2 IU FSH (Gonal F, Serono, Solna, Sweden). Without any oil cover follicles were cultured in a humidified incubator gassed with 5% CO₂ in air at 37°C.

[0088] Start of culture was defined as day 0. Culture medium was exchanged every other day. The diameter of the follicles was measured each day using x 100 magnification and a calibrated micrometer. In addition, the survival rate of the follicles was checked by evaluation of degeneration (darkening of the follicle) and bursting (loss of the oocyte).

The culture time was 4 days.

[0089] At day 2 and day 3 of culture test compound or vehicle at a volume of 1.72 µl was added to the culture medium. Test compounds were dissolved in ethanol (vehicle).

Examination of follicles

[0090] Oocytes arrested in meiosis are characterised by an intact nucleus with a prominent nucleolus, known as germinal vesicle (GV). Upon reinitiation of meiosis the nucleolus and the nuclear envelope disappear and this is characterised by a breakdown of the GV, which then is called germinal vesicle breakdown (GVB).

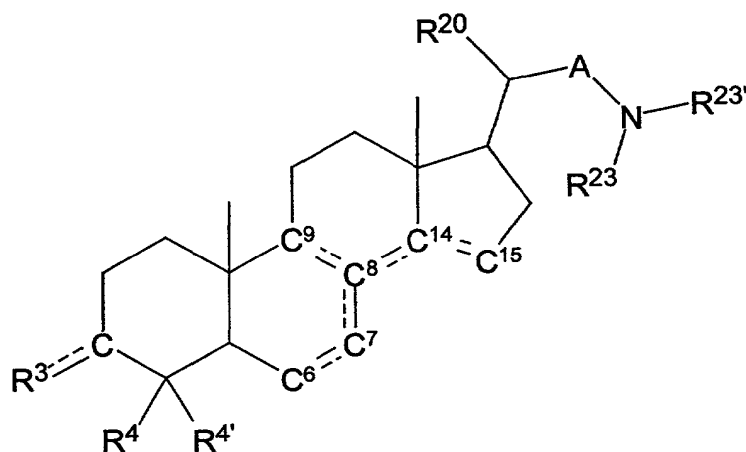
[0091] At day 4, by the end of the culture period, follicles were checked for resumption of meiosis. The number of follicles having oocytes with germinal vesicle (GV) or germinal vesicle breakdown (GVB) was counted using a stereo microscope or an inverted microscope with differential interference contrast equipment. The percentage of follicles with GVB per total number of follicles was calculated in the test cultures and compared to the vehicle control culture.

Table 2

Activation of oocyte maturation in the mouse follicular culture system			
Compounds	Follicles [n]		Activation [%]
	GV	GVB	GVB
Control (1,72 ethanol))	16	2	11
10 µM FF-MAS	9	0	0
1 µM compound example 1	2	7	78
3 µM compound example 1	0	9	100
10 µM compound example 1	0	8	100
GV = oocytes with a germinal vesicle GVB = oocytes with germinal vesicle breakdown n = number of follicles			

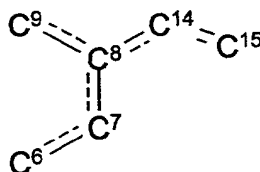
Claims

1. A steroid compound of general formula



I

wherein in the moiety



IA

each bond between C⁶ and C⁷, between C⁷ and C⁸, between C⁸ and C⁹, between C⁸ and C¹⁴ and between C¹⁴ and C¹⁵, independently, may be a single bond or a double bond, with the proviso that each carbon atom C⁶, C⁷, C⁸, C⁹, C¹⁴ and C¹⁵ is bonded to each neighbouring C atom by a single bond or at the most by one double bond, CR³ is

a) C=O or
b) CH-OR³, wherein R³ is selected from the group comprising hydrogen, unsubstituted or substituted, linear or branched C₁ - C₁₀ alkyl and C(O)-R³, bonded to the CH-O moiety via the C(O) moiety, wherein R³ is selected from the group comprising

- i) substituted or unsubstituted, linear or branched C₁ - C₁₀ alkyl,
 - ii) substituted or unsubstituted, linear or branched C₁ - C₁₀ fluoro alkyl,
 - iii) unsubstituted or substituted C₁ - C₁₀ aryl,
 - iv) unsubstituted or substituted C₁ - C₁₀ heteroaryl,
 - v) unsubstituted or substituted, linear or branched C₁ - C₁₀ alkyloxy and
 - vi) unsubstituted or substituted, linear or branched C₁ - C₁₀ alkylamino, or
- c) CH-SO₂-R³ or C=NOR³, wherein R³ has the same meaning as above, or
d) CH-O-R³, wherein R³ is unsubstituted or substituted, linear or branched alkyl and forms a cyclic ether both with the C atom of the steroid skeleton and the O atom, or
e) a cyclic ring structure with the C³ atom, wherein R³ is unsubstituted or substituted, linear or branched C₂ - C₁₀ alkyl, or
f) CH-Hal, wherein Hal is F, Cl, Br or I,

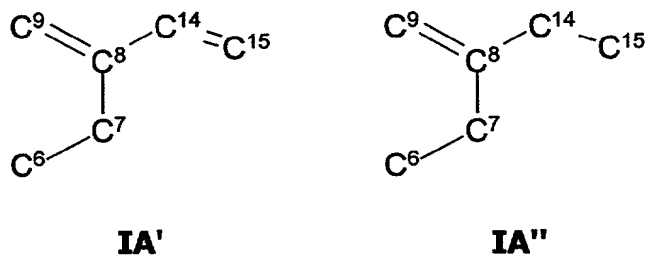
R⁴, R^{4'} and R²⁰, independently, are selected from the group comprising hydrogen and unsubstituted or substituted, linear or branched C₁ - C₄ alkyl,

R²³ and R^{23'}, independently, are selected from the group, comprising hydrogen, unsubstituted or substituted, linear or branched C₁ - C₈ alkyl and unsubstituted or substituted, linear or branched C₁ - C₈ alkenyl and unsubstituted or substituted, linear or branched C₁ - C₈ alkyl, at least one of the alkyl carbon atoms being substituted by any of O, N and S and unsubstituted or substituted, linear or branched C₁ - C₈ alkenyl, at least one of the alkenyl carbon atoms being substituted by any of O, N and S and unsubstituted or substituted, linear or branched C₆ - C₁₀ aryl, or

R²³ and R^{23'} together form an unsubstituted or substituted, linear or branched C₁ - C₇ alkyl spacer or an unsubstituted or substituted, linear or branched C₁ - C₇ alkyl spacer, at least one of the alkyl carbon atoms being substituted by any of O, N and S,

A is a single bond or an unsubstituted or substituted methylene or ethylene spacer.

2. The steroid compound according to claim 1, wherein the moiety I has one of general formulae



3. The steroid compound according to claim 1 or claim 2, wherein CR³ is CH-OH or CH-O-C(O)-R³.
4. The steroid compound according to any one of the preceding claims, wherein R³ is an ester radical of a monocarboxylic acid, a dicarboxylic acid or of an inorganic acid.
5. The steroid compound according to claim 4, wherein R³ is (CH₂)_n-COOH, wherein n = 1, 2, 3, 4, 5 or 6.
6. The steroid compound according to any one of claims 4 and 5, wherein R³ is acetyl, propionyl, pivaloyl, butanoyl, benzoyl, nicotinyl, isonicotinyl, hemi succinoyl and hemi glutaroyl.
7. The steroid compound according to claim 3, wherein R³ is fluoromethyl.
8. The steroid compound according to any one of the preceding claims, wherein R⁴ and R^{4'}, independently, are hydrogen or methyl.
9. The steroid compound according to any one of claims 1 to 7, wherein R⁴ and R^{4'}, independently, are C₁ - C₄ alkyl, substituted by halogen, hydroxy, alkoxy or aryloxy.
10. The steroid compound according to any one of the preceding claims, wherein R²⁰ is hydrogen or methyl.
11. The steroid compound according to any one of the preceding claims, wherein R²³ and R^{23'}, independently, are C₁ - C₄ alkyl or C₁ - C₄ alkenyl, substituted by at least one radical, selected from the group, comprising linear or branched C₁ - C₄ alkyl and C₁ - C₄ alkoxy.
12. The steroid compound according to any one of the preceding claims, wherein R²³ and R^{23'} together with the amino nitrogen form a nitrogen containing heterocyclic ring structure, selected from the group, comprising piperidin-1-yl, morpholin-4-yl, piperazin-1-yl, pyrrolidin-1-yl, pyridin-1-yl, chinolin-1-yl, isochinolin-1-yl, pyridazin-1-yl, pyrimidin-1-yl, pyrazin-1-yl, pyrrol-1-yl, indol-1-yl, chinoxalin-1-yl, pyrazol-1-yl, imidazol-1-yl, thiazol-1-yl and oxazol-3-yl ring structures and substituted derivatives thereof.
13. The steroid compound according to claim 12, wherein the ring structure is a moiety, selected from the group comprising piperidin-1-yl, morpholin-4-yl, piperazin-1-yl and pyrrolidin-1-yl.
14. The steroid compound according to any one of claims 12 and 13, wherein the ring structure is substituted with a radical, selected from the group comprising alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, alkylcycloalkyl, aryl, alkylaryl, hydroxy, alkoxy, alkylcycloalkoxy, alkyloxycycloalkyl, alkylaryloxy, alkyloxyaryl, halogen and acyl.
15. The steroid compound according to any one of the preceding claims, wherein A is methylen.
16. The steroid compound according to claim 1, selected from the group comprising
 (20S)-20-[(3,3-dimethylpiperidin-1-yl)methyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol,
 (20S)-20-[(piperidin-1-yl)methyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol,
 (20S)-20-[(4,4-dimethylpiperidin-1-yl)methyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol,
 (20S)-20-[(4-methylpiperazin-1-yl)methyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol,

(20S)-20-[(4-phenylpiperazin-1-yl)methyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol,
 (20S)-20-[(morpholin-4-yl)methyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol, (20S)-20-[(4-(pyrimidin-2-yl) piper-
 azin-1-yl)methyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol,
 (20S)-20-[(pyrrolidin-1-yl) methyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol,
 (20S)-20-[(3,3-dimethylpiperidin-1-yl) methyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol hemisuccinate,
 (20S)-20-[N-(3-methoxypropyl)aminomethyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol,
 (20S)-20-aminomethyl-4,4-dimethyl-5 α .pregna-8,14-dien-3 β -ol,
 (20S)-20-[N,N-di-(2-methoxyethyl)aminomethyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol,
 (20S)-20-[N-(2,2-dimethylethylen)aminomethyl]-4,4-dimethyl-5 α -pregna-8,14-dien-3 β -ol.

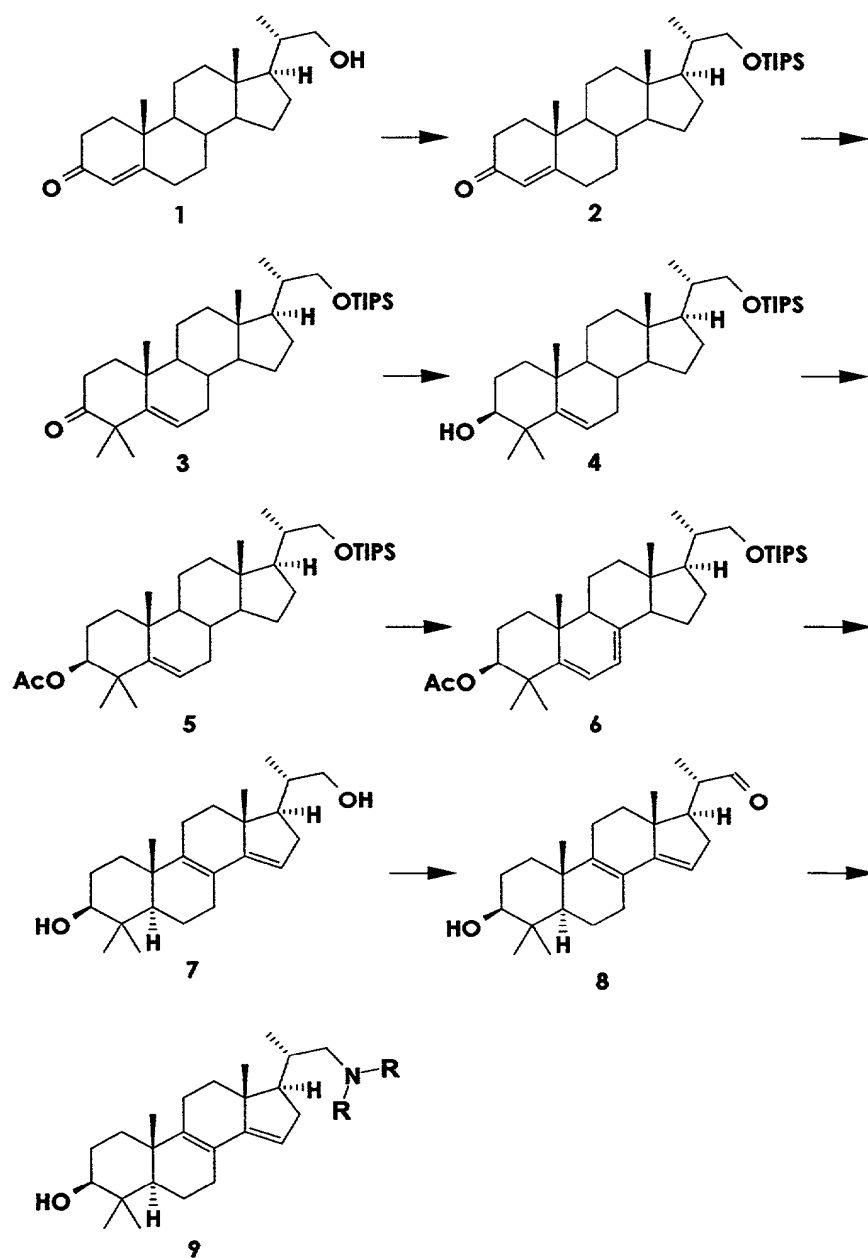
17. A pharmaceutical composition comprising at least one steroid compound of general formula I according to any one of claims 1 to 16 and at least one pharmaceutically acceptable excipient.

18. A use of steroid compounds of general formula I according to any one of claims 1 to 16 for the preparation of a meiosis-regulating medicament.

19. A method of regulating meiosis comprising administering to a subject in need of such a regulation an effective amount of at least one steroid compound of general formula I according to any one of claims 1 to 16.

20. A method for the preparation of steroid compounds of general formula I according to any one of claims 1 to 16, wherein R⁴ and R^{4'} are unsubstituted or substituted, linear or branched C₁ - C₄ alkyl, comprising

- a. starting from (20S)-20-hydroxymethyl-pregn-4-en-3-one,
- b. introducing two alkyl groups in position 4 by alkylation,
- c. reducing the keto group to a hydroxy group,
- d. introducing a Δ^7 double bond by bromination/dehydrobromination,
- e. isomerizing the dien $\Delta^{5,7}$ to the dien $\Delta^{8,14}$ by heating in the presence of acid,
- f. oxidizing the 17-hydroxy group to an aldehyde group and
- g. reductively aminizing the aldehyde group.

Scheme 1**Fig. 1**

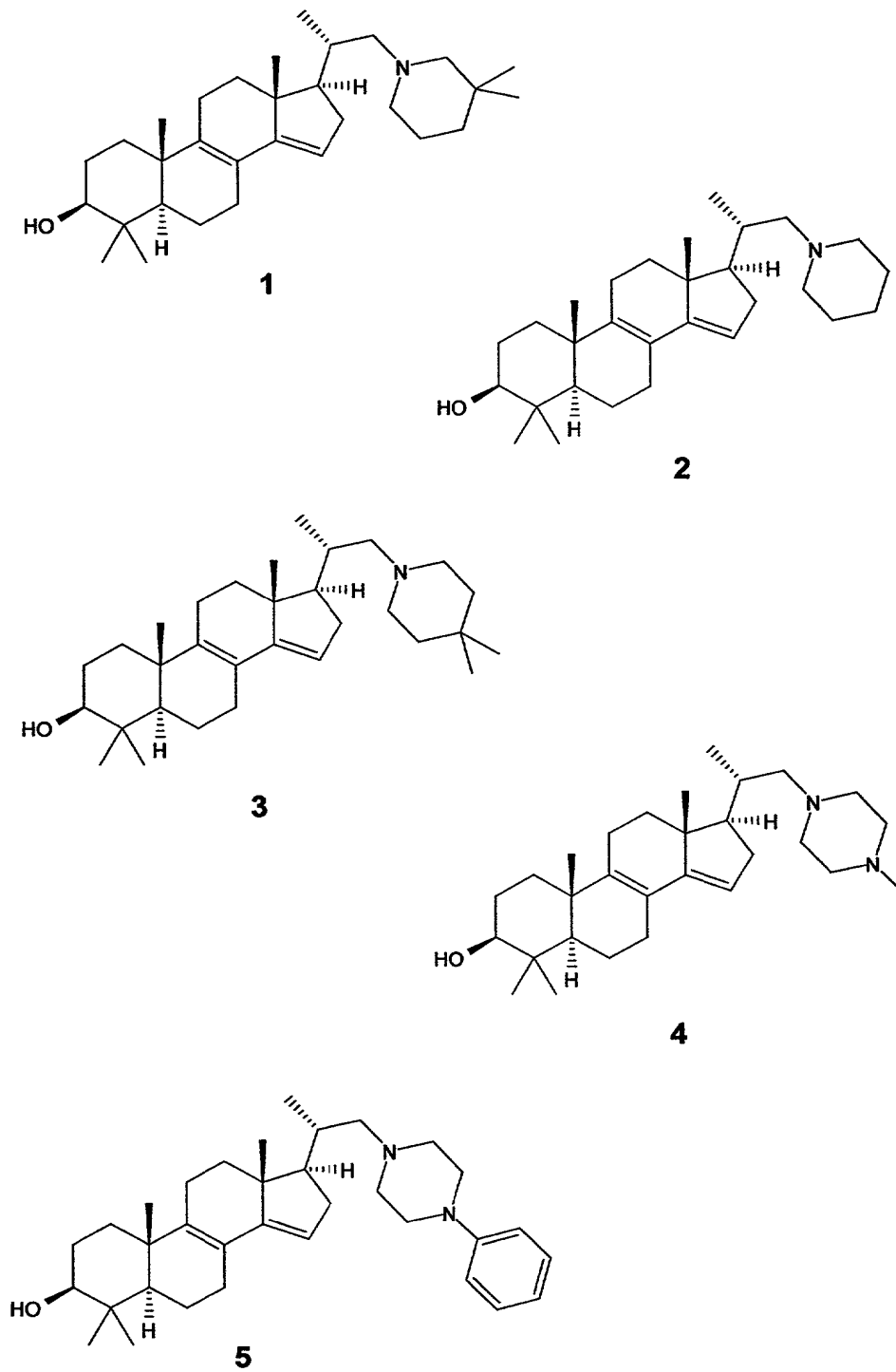


Fig. 2A

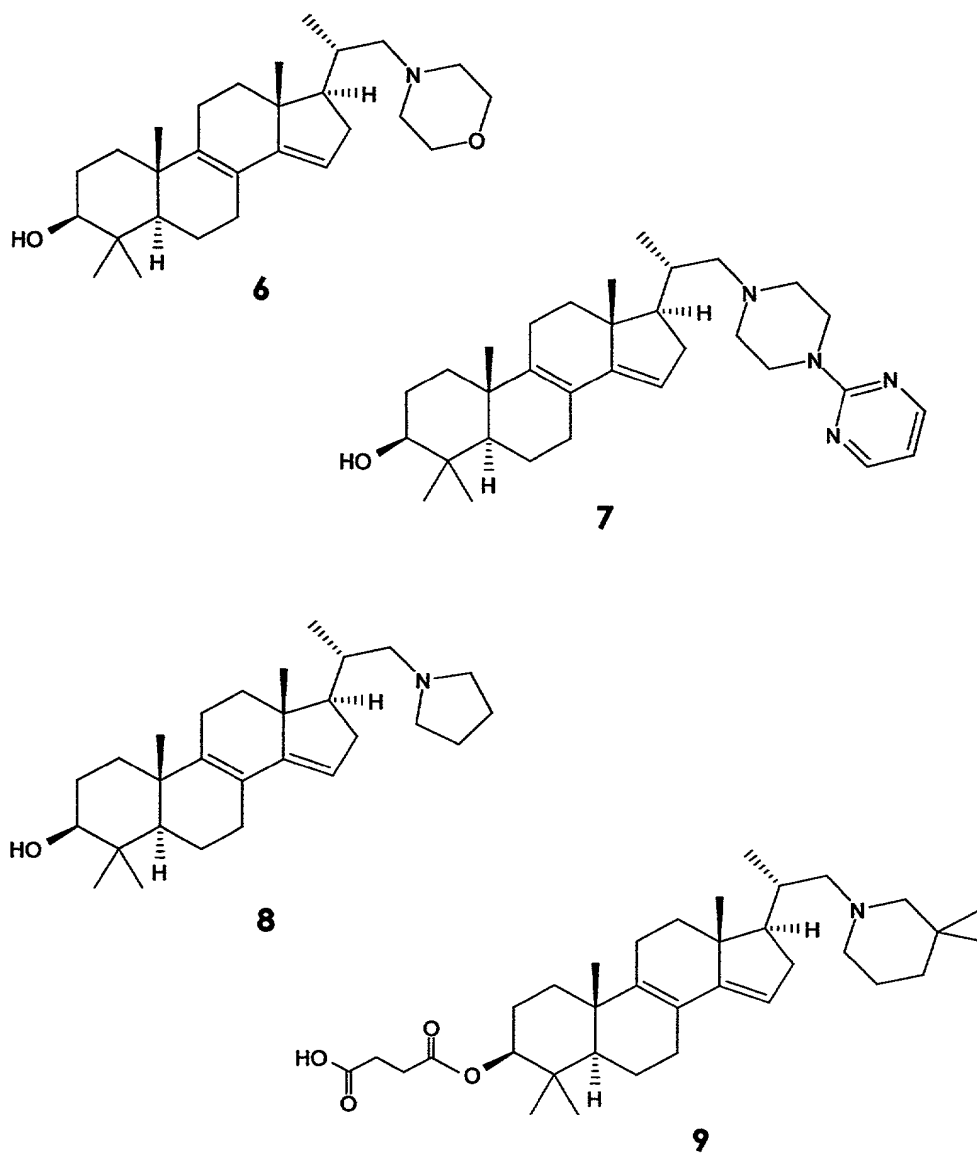


Fig. 2B

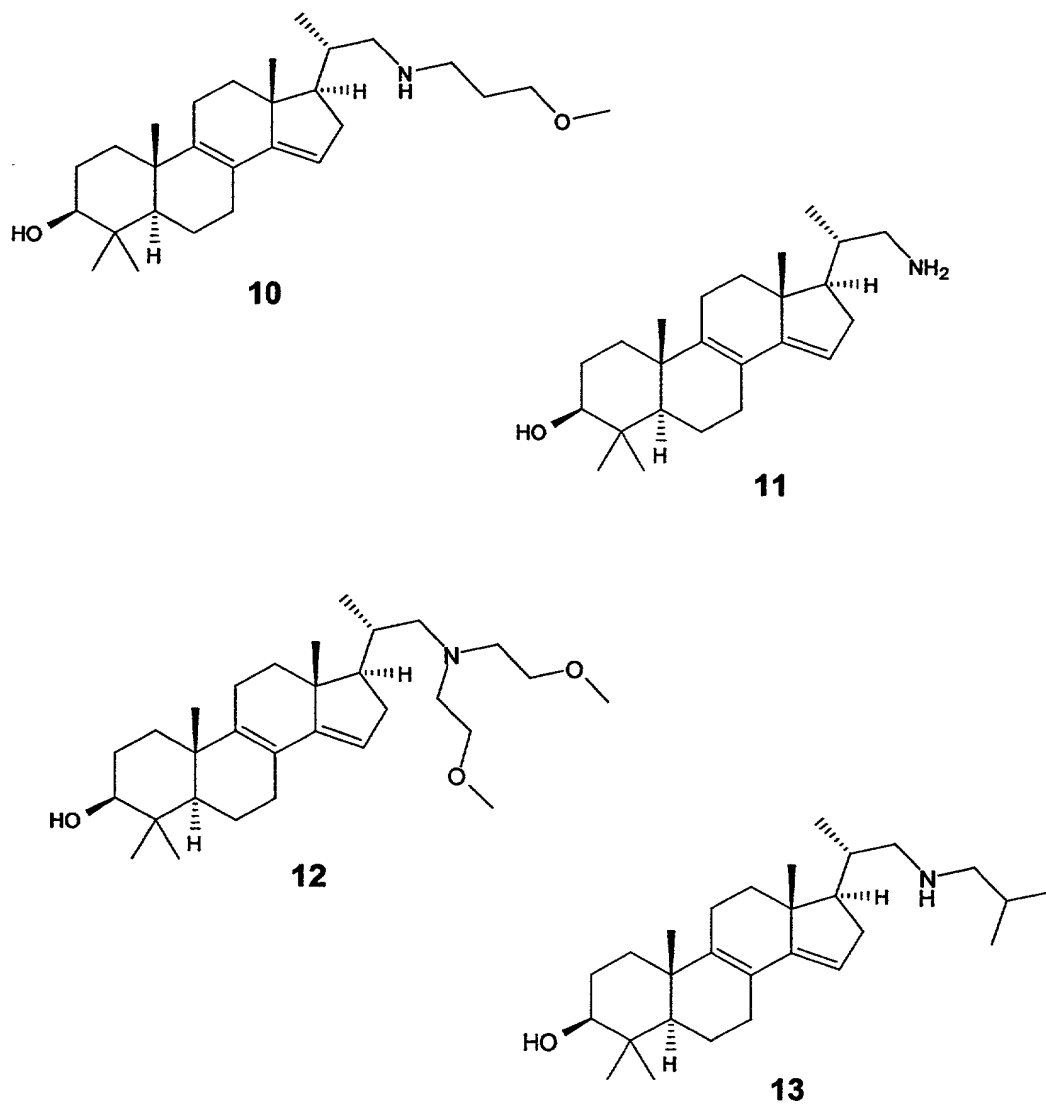


Fig. 2C



European Patent
Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

EP 01 25 0108

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	WO 96 27658 A (NOVONORDISK AS ; GUDDAL ERLING (DK); BYSKOV ANNE GRETE (DK); GROENV) 12 September 1996 (1996-09-12) * page 2, line 29 - page 3, line 11 * * page 5, line 22-29 *	1-20	C07J43/00 C07J41/00 A61K31/58 A61K31/57 A61P15/08
X	WO 99 58549 A (BLUME THORSTEN ; BREINHOLT JENS (DK); FAARUP PETER (DK); MURRAY ANT) 18 November 1999 (1999-11-18) * page 19, line 7,8; claim 3 *	1-20	
X	US 3 419 661 A (ELDER WILLIAM H) 31 December 1968 (1968-12-31) * column 1, line 68-71 * * column 2, line 4-20 *	1-20	
X	LU, MATTHIAS C. ET AL: "Inhibition of cholesterol side-chain cleavage. 4. Synthesis of A or B ring-modified azacholesterols" J. MED. CHEM. (1981), 24(9), 1038-42 , XP002175968 * page 1039; examples 1B,1E,1F * * page 1040; table 1 *	1,3,8,10	
			TECHNICAL FIELDS SEARCHED (Int.Cl.7)
			C07J A61K A61P
INCOMPLETE SEARCH The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims. Claims searched completely : Claims searched incompletely : Claims not searched : Reason for the limitation of the search: see sheet C			
Place of search THE HAGUE		Date of completion of the search 28 August 2001	Examiner Watchorn, P
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	

EP0 FORM 1503 03/82 (P04007)



European Patent
Office

INCOMPLETE SEARCH
SHEET C

Application Number
EP 01 25 0108

Although claim 19 is directed to a method of treatment of the human/animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the compound/composition.

Claim(s) searched completely:
2,12-14,16

Claim(s) searched incompletely:
1,3-11,15,17-20

Reason for the limitation of the search:

The initial phase of the search revealed a very large number of documents relevant to the issue of novelty. So many documents were retrieved that it is impossible to determine which parts of the claim(s) may be said to define subject-matter for which protection might legitimately be sought (Article 84 EPC). For these reasons, a meaningful search over the whole breadth of the claim(s) is impossible. Consequently, the search has been restricted to the 8-ene and 8,14-diene compounds of claim 2 and the cyclic-amine derivatives of claims 12-14. The search on the pharmaceutical compositions, uses employing the compounds of claim 1 (claims 17-19) and on the process for their production (claim 20) has been limited in the same way.



European Patent
Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 01 25 0108

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X	DAVIS, M. ET AL: "Steroid amines. V. 20-(1-Pyrrolidinyl)pregnane derivatives" J. CHEM. SOC., PERKIN TRANS. 1 (1972), (11), 1420-4 , XP002175969 * page 1423, column 2, paragraph 7 * * page 1424, column 1, paragraph 4 * * page 1424, column 2, paragraph 4 *	1,3,8, 10,12,13	
X	SHEETS J J ET AL: "Active site-directed inhibitors of cytochrome P-450sc. Structural and mechanistic implications of a side chain-substituted series of amino-steroids" JOURNAL OF BIOLOGICAL CHEMISTRY. (MICROFILMS), AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 258, no. 19, 10 October 1983 (1983-10-10), pages 11446-11452, XP002104638 * page 11448, column 2; table I *	1,3,8, 10,15	TECHNICAL FIELDS SEARCHED (Int.Cl.7)
X	US 3 535 312 A (PHILIPPSON RAINER ET AL) 20 October 1970 (1970-10-20) * example 10 *	1,3,4,6, 8,10,12, 13,15	
X	MANGLA, A. T. ET AL: "Sterol C-methyl transferase from Prototheca wickerhamii mechanism, sterol specificity and inhibition" BIOORG. MED. CHEM. (2000), 8(5), 925-936 , XP001023708 * page 930; figure 5; example 27; table 2 *	1-3,8,10	
	---	-/--	

EPO FORM 1503 03.82 (P04C10)

**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.**

EP 01 25 0108

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

28-08-2001

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9627658 A	12-09-1996	AU 717180 B	16-03-2000
		AU 4784596 A	23-09-1996
		BR 9607673 A	16-06-1998
		CA 2214126 A	12-09-1996
		CZ 9702797 A	14-01-1998
		EP 0813595 A	29-12-1997
		HU 9800731 A	28-07-1998
		JP 11501806 T	16-02-1999
		NO 974089 A	05-09-1997
		PL 322106 A	05-01-1998
		US 5830757 A	03-11-1998
WO 9958549 A	18-11-1999	EP 0957108 A	17-11-1999
		AU 3701199 A	29-11-1999
		BR 9910415 A	09-01-2001
		EP 1077992 A	28-02-2001
		NO 20005667 A	12-01-2001
		US 2001003782 A	14-06-2001
US 3419661 A	31-12-1968	NONE	
US 3535312 A	20-10-1970	AT 281315 B	25-05-1970
		AT 287936 B	10-02-1971
		AT 290026 B	15-03-1971
		BE 710447 A	07-08-1968
		CH 534145 A	28-02-1973
		CH 528497 A	30-09-1972
		CH 533609 A	15-02-1973
		DE 1643004 A	08-04-1971
		DK 126112 B	12-06-1973
		FR 1574709 A	18-07-1969
		GB 1217913 A	06-01-1971
		NL 6801733 A	08-08-1968
		SE 342617 B	14-02-1972
		DE 1668684 A	23-09-1971
US 3342811 A	19-09-1967	BE 656614 A	01-04-1965
		DE 1493009 A	07-08-1969
		FR 1458087 A	23-01-1967
		GB 1095167 A	13-12-1967
		NL 6414157 A	07-06-1965

EPO FORM P3489

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82